General Disclaimer

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.

- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.

- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.

- This document is paginated as submitted by the original source.

- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

Quarterly Status Report No. 12

"Specific destruction of the O_2 evolving reactions and the Photorestoration of O_2 Evolution"

April 6, 1970 - July 21, 1970

under
Contract NASW-1592
National Aeronautics and Space Administration
Washington, D. C. 20546

Research Institute for Advanced Studies
(RIAS)
Martin Marietta Corporation
1450 South Rolling Road
Baltimore, Maryland 21227

SPECIFIC DESTRUCTION OF THE O_2 EVOLVING REACTIONS AND THE PHOTORESTORATION OF O_2 EVOLUTION

Previously we reported briefly on some of the effects of hydroxylamine and related compounds upon the System II complex. Qualitatively the overall effect of extraction of chloroplasts or several different algae with these compounds is simple. In darkness, the O_2 evolving reactions are "irreversibly" destroyed without affecting the System II quantum trapping centers. Proof for this latter statement has been recently obtained. Quantum requirement measurements for O_2 evolution have been made before and after such extractions. Without extraction of spinach chloroplasts, 10-11 $h\nu$ (640nm) per O_2 were required. After 5-10 min extraction of these chloroplasts with hydroxylamine (10mM), the quantum requirement became 90-110 $h\nu/O_2$. Nevertheless such extracted chloroplasts photooxidized NH_2OH or Mn^{+2} through Systems I and II with high quantum efficiency ($2h\nu$ and $4h\nu$/ equiv., respectively) at wave lengths predominately exciting System II. Since these photooxidations were inhibited by DCMU and showed a quantum yield profile similar to that for photosynthesis, we are assured that the extraction affects nothing more than the O_2 yielding reactions and the associated Mn catalysts.

Despite rather extensive studies on the kinetics of this annihilation of the O_2 yielding reactions and the specificity of functional groups of molecules inducing this effect, we still are lacking a total explanation for the mechanism of the NH_2OH effect.

Basically the induced decay of the O_2 yielding reactions in chloro-

plasts or algae follows simple first-order kinetics with the rate constant proportional to the concentration of NH_2OH: the addition of FeCN, ascorbate, or ascorbate-DCIPH_2 does not change the rate constant; thus we conclude that the hydroxylamine effect is independent of the redox buffering limits of these reagents.

This decay rate constant is pH dependent in a manner suggesting that the active species is NH_2OH and not NH_3OH^+. However, with related compounds, including isosteric compounds with similar or greater chemical reactivity than NH_2OH, this relationship does not hold. For instance, all substituted derivatives (O-methyl, N-methyl, O-sulfonate) of NH_2OH show varying effectiveness in causing loss of the Mn catalyst and O_2 evolution, yet we have been unable to specifically relate the observed effect to one and only one chemical property of such compounds. Moreover, hydrazine (NH_2-NH_2) which is isosteric and more reducing than hydroxylamine is virtually ineffective in promoting the loss of O_2 yielding reactions and Mn. From these and other studies we can only conclude at present that the specificity is linked to the polar -OH (or $-OSO_3^-$) functional group of hydroxylamine. Unknown parameters, possibly properties of chloroplast membranes, or of the Mn-catalyst itself, may be involved.

As was observed with Mn deficient algae, illumination of NH_2OH extracted cells results in restoration of the O_2-evolving reactions. Though the effect of photorestoration is more dramatic (15 to 20 fold increases in quantum yield) than could be obtained with Mn deficient tissue obtained by growth (12), the process appears essentially similar, irrespective of the type of alga. Thus, the wavelength sensitization, kinetics of the process, and inhibitor studies all point to the same result — namely, a rebinding and incor-

poration of Mn into the O_2 yielding catalyst. The failure to achieve photorestoration with Mn deficient or extracted chloroplasts remains a dilemma.

In addition, we have studied the marked sensitivity of hydroxylamine extracted chloroplasts to photoinhibition. The intensity relationship for this photoinhibition saturates in the same manner as photosynthesis itself. Though considerable information (kinetics, quantum yield, etc.) have been obtained we cannot as yet offer a detailed mechanism to explain all data. The end result of this photoinhibition is the loss of the DCMU variable flourescence and System II sensitized photooxidations of artificial electron donors. Photosystem I reactions are not affected.

Fast processes in Photosynthesis.

Previous reports have indicated the development of an instrument capable of optical measuremnts in the time range of tens to hundreds of microseconds. This time range is intermediate between the times for electronic excitation transfer and those for collision chemistry; i.e., processes in this time range are those that do not require transport of molecules in solution and include the initial steps in the transduction and storage of energy.

The instrument was designed to look at optical changes shortly after the photosynthetic system is perturbed by a short flash. A rotating disc blocks the direct effects of the flash from the detecting photomultiplier. Luminescence and optical density changes can be observed after the flash. The relaxation of the system after the perturbing flash can thus be studied in order to infer how the system works.

The present system can measure the fluorescence from a weak measuring beam after a saturating flash. Preliminary measuremnts indicate the occurance of changes in the fluorescence yield in the time range studied, i.e; a few hundred microseconds. The apparatus is now being improved to increase the sensitivity. Measurements of changes in fluorescence yield are in progress. These will be reported on at some later date. In addition the apparatus is being further developed to allow measurements of changes in optical density produced by the perturbing flash.

A number of measurements have been made on the delayed luminescence from chloroplasts and algae. Delayed luminescence (D.L.) represents energy which is stored in the photosynthetic system and later reemitted with the

same spectra as the fluorescence.

Briefly the measurements are made by illuminating the sample with a brief (approx. 3 μsec) saturating flash and observing the luminescence following the flash. Observations can be made at any time following 50 secs after the flash. The observations to date indicate the following:

a) A component of delayed luminescence decays with a half time of about 50 μseconds at 20°C;

b) The magnitude of the component depends on the state of the photosynthetic system;

c) To be produced, the luminescence requires oxidized acceptor of system II during the flash;

d) The total energy emitted in this component is, in the presence of DCMU, about 10^{-4} quanta/per quantum given to a trap. The energy emitted under other conditions is somewhat less.

e) The amount of energy in this component does not vary much with temperature; the half time for decay varies by a factor of two for a change of about 20°C.

f) The D.L. yields from a sequence of flashes vary. As was mentioned in our previous report, there is an oscillation similar to and undoubtedly reflecting the oscillations of oxygen production in a sequence of flashes. These patterns of oscillation can be altered chemically.

The fact that the delayed luminescence varies with the state of system II indicates that it is closely related to the primary photochemical acts of system II. Since delayed luminescence depends not only on the amount of energy stored which is later used to bring the chlorophyll to the singlet

excited state, but also on the probability of it being emitted once in the excited state, the storage process can only be understood if the probability of emmission is known. For example, the oscillations can occur either in the storage process or in the emmission process. Since fluorescence is a measure of the latter, interpretation of delayed emission will have to await measurements of fluorescence which are now in progress.

Measurements of temperature effects, total yields as well as chemical affects of both delayed emmission and fluorescence should allow us to determine the significance of the delayed emmission.

REFERENCES

1. Cheniae, G. M. and I. F. Martin. Biochem. Biophys. Res. Commun. 28: 89 (1967).

2. Cheniae, G. M. and I. F. Martin. Plant Physiol. 44: 351 (1969).